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Myelodysplastic syndrome(MDS)の
造血障害に関する研究

大森聖一

The Mechanism of Prostaglandin E₂ Effects on Hemopoietic Progenitor Cells from Human Bone Marrow

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MDS-macrophage derived inhibitory activity on myelopoiesis of MDS abnormal clones

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Abstracts

We studied the effect of myelodysplastic syndrome (MDS)-derived adherent cells on colony formation of granulocyte-macrophage progenitors (CFU-GM) in both normal and MDS bone marrow cells. MDS-adherent cells suppressed the growth of normal CFU-GM colony formation. Antibodies against ferritin almost totally neutralized the haematopoietic inhibitory activity. Antibody against gamma-interferon (γ -IFN) did not have such effect.

By cytogenetic analysis using G-staining method, MDS-derived CFU-GM colony showed abnormal clones. MDS have been recognized to be a mosaic of normal and abnormal clones. MDS-macrophages suppressed the growth of progenitor cells derived from normal clones by soluble factors, but did not suppress the growth of those from abnormal clones. It is suggested that progenitor cells derived from abnormal clones are freed from the negative myelopoietic regulator, that may be related to the progress of leukemia.

Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous group of haemopoietic disorders characterized by cytopenias (Jacobs,1985; Yoshida,1987). It has been reported that MDS-derived inhibitory macrophage (Ohmori et al,1990), MDS-monocyte-derived lipid containing macrophage (MDLM)(Ohmori et al,1992) and MDS derived mononuclear cells (Fukuoka et al,1987; Cukrova et al,1989 ,1990) inhibit the growth of normal CFU-GM colony formation. Macrophages have been recognized to have a number of important biological activities in the regulation of the haemopoiesis, able to produce haemopoietic growth factors inducing granulocyte colony stimulating factor (G-CSF) , granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin 1 (Il-1) (Fibbe et al,1980, 1988). However, in contrast to positive effects on haemopoiesis, macrophages have also been shown to release of inhibitory molecule such as tumor necrosis factor (TNF), prostaglandin E₂ (PGE₂), γ -IFN (Peluse et al,1981; Paukovits et al,1991).

It was the aim of the present study to investigate the in vitro effects of MDS-macrophage on haematoprogenitor cells to know the interaction between colony stimulating and inhibitory factors and progenitor cells.

Materials and Methods

<Patients>

Bone marrow cells were obtained from patients with MDS[refractory anemia (RA):1, chronic myelo-monocytic leukemia (CMML):1, refractory anemia with excess of blasts (RAEB):1, RAEB in transformation (RAEB-t):1]. The patients were classified according to the FAB criteria (Bennett et al,1982). The clinical characteristics of the MDS patients are summarized in Table 1.

<Preparation of Medium Conditioned(CM) by Macrophages>

Light density cells were separated by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway,N.J.) density gradient centrifugation at 400g for 30 min, washed three times and resuspended in Iscove's modified Dulbecco's medium (IMDM, GIBCO, Grand Island, N.Y.). Cells were further separated into non-adherent and adherent cells after incubation in plastic culture dishes (Miles Scientific, Naperville,Ill) at 37°C for 90 min in an atmosphere of 5% CO₂. Non-adherent cells were collected by gently swirling the dishes and carefully aspirating the floating cells (Mosier et al,1967). Adherent cells incubated for 24 h in a 5% CO₂ incubator were >90% monocytes showed strong positiveness in non-specific elastase staining. The cell-free supernatant(CM), obtained by centrifugation, were sterilized using Millipore membrane(0.45 µm)(Millex-HA,Japan Millipore Ltd,Japan) and stored at -20°C until use. They are referred to as macrophage-CM and were used as effector in the CFU-GM assay.

<CFU-GM>

CFU-GM cultures were carried out according to the method originally described by Iscove(Iscove et al,1971). Briefly, 1×10^5 /ml non-adherent bone marrow mononuclear cells (BM-MNC)were incubated in IMDM containing 0.87% methylcellulose, 20% fetal calf serum(FCS)(Irvine Scientific, Santa Ana, Calif.)

and 10ng/ml human recombinant GM-CSF(Sumitomo Chemicals, Tokyo). Colonies (more than 40 cells) were scored under an inverted microscope after 8 days of incubation.

< Effect of MDS-macrophage-CM on CFU-GM colony formation>

MDS-macrophage-CM derived above were tested for their effect on CFU-GM colony formation at a concentration of 10 % in the CFU-GM assay system.

<Inactivation of inhibitory activity by antibodies upon MDS-macrophage-CM>

CFU-GM cultures were set up with normal BM-MNC as described above and these were supplemented with the following: (1)MDS-macrophage-CM alone, (2)MDS-macrophage-CM plus anti-ferritin antibody (specific rabbit serum against human placental ferritin purified by DEAE column chromatography,Cosmo Bio, Japan), (3)MDS-macrophage-CM plus anti-PGE₂ antibody, (4)MDS-macrophage-CM plus anti-TNF antibody, (5)MDS-macrophage-CM plus anti γ -IFN antibody, (6)MDS-macrophage-CM plus anti ferritin, anti PGE₂, anti TNF and anti γ -IFN antibodies. The diluted antibodies had no deleterious effect on normal CFU-GM growth. Ferritin used in this assay exhibited one band on gradient polyacrylamide gel electrophoresis.

<Statistical analysis>

In some experiments, statistical analysis was performed utilizing the Students' t-test.

Results

Effects of MDS-macrophage-CM on normal CFU-GM

Effects of MDS-macrophages-CM are shown in Fig 1. MDS-macrophages-CM suppressed the growth of normal CFU-GM ($p < 0.05$ as compared with CFU-GM cultured in the absence of MDS-macrophage-CM)

Effects of MDS-macrophage-CM on MDS-CFU-GM.

Effects of MDS-macrophage-CM are shown in Fig 2. MDS-macrophage-CM did not suppress the growth of auto and allo MDS-CFU-GM.

These experiments were performed three times using five normal BM-MNC and four MDS BM-MNC as the target CFU-GM.

Effect of anti ferritin, anti PGE_2 , anti TNF and anti γ -IFN antibodies on CFU-GM inhibitory activity of MDS-macrophage-CM.

Effects of those antibodies are shown in Fig 3. The inhibitory effect in MDS-macrophage-CM was almost neutralized by anti-ferritin antibody. Anti- γ -IFN antibodies did not neutralize the inhibitory activity.

Chromosomal analysis

In CFU-GM colony assay, colonies picked up by Pasteur pipette on day 7 and cytogenetic analysis was performed using G-staining method. All colonies derived from MDS-CFU-GM showed abnormal clonality.

Discussion

MDS is characterized by a diversity of phenotypic manifestations with varying degrees of ineffective hemopoiesis with impaired normal haemopoiesis and a high probability of eventual leukemic change (Colombat et al,1988).

These are two mechanisms responsible for impaired normal haemopoiesis, 1) presence of inhibitory activity derived from MDS bone marrow cell (Fukuoka et al,1987; Cukrova et al,1989) MDS-inhibitory macrophage (Ohmori et al,1990) and MDS-MDLs (Ohmori et al,1992), 2) defects in MDS progenitors to proliferate and differentiate as a result of stem cell abnormality.

In the field of haemopoiesis, monocyte-macrophages have an important role by secreting stimulating factors such as IL-1, G-CSF, GM-CSF (Nicola et al,1986; Gold et al,1972) and inhibitory factors such as PGE₂, ferritin, TNF, γ -IFN and transforming growth factor (TGF)- β (Kurland et al,1981; Murase et al,1987; Pelus et al,1981,1988; Broxmeyer et al,1982,1989).

We focused on the modulating properties of MDS-macrophages. The data showed that MDS-macrophage-CM suppressed normal CFU-GM colony formation. The inhibitory activity was neutralized mainly by anti-ferritin antibody and partially by anti-PGE₂, anti-TNF antibodies, suggesting that the inhibition was PGE₂, TNF, and ferritin mediated. Other mechanisms possible of macrophage derived inhibitory activity include TGF- β (Sing et al,1988) and macrophage inflammatory protein (MIP)-1 α .

On the other hand, MDS-macrophage did not suppress the growth of MDS-CFU-GM colony formation. By examining of chromosomal change for MDS-CFU-GM colonies, MDS-colonies contained abnormal clones. Therefore the progenitor cells originated from abnormal clones are not influenced by the negative regulation of myelopoiesis.

MDS-macrophage might be destined to produce negative regulator such as PGE₂, ferritin and TNF greater than normal macrophages on genetic level

and suppress the normal growth of CFU-GM.

MDS are a mosaic of normal and abnormal clones. Progenitors derived from normal clones are suppressed by inhibitory factors, and progenitors derived from abnormal clones are not suppressed. It was suggested that abnormal clone cells gain growth advantage over normal cellular elements by either an inhibitory effect on normal haematopoietic progenitors or by a direct stimulation of the leukemic clone on cytogenetic level related to a greater tendency toward overt leukemia.

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Figure Legends

Fig 1.

Effect of MDS-macrophage-CM on normal CFU-GM.

MDS-macrophage-CM from patients were added to 1×10^5 normal BM-MNC at a concentration of 10% in the culture medium. Data are represented as mean \pm SE of control growth (without MDS-macrophage-CM). (Control colonies = 128.7 ± 6.8 .)

Fig 2

Effect of MDS-macrophage-CM on MDS-CFU-GM.

MDS-macrophage-CM from patients were added to the culture system of 1×10^5 MDS-BM-MNC at a concentration of 10%. (A: auto MDS-BM-CM, B: allo MDS-BM-CM.) Data are represented as mean \pm SE percent of control growth. (Control colonies = 34.0 ± 3.1 .)

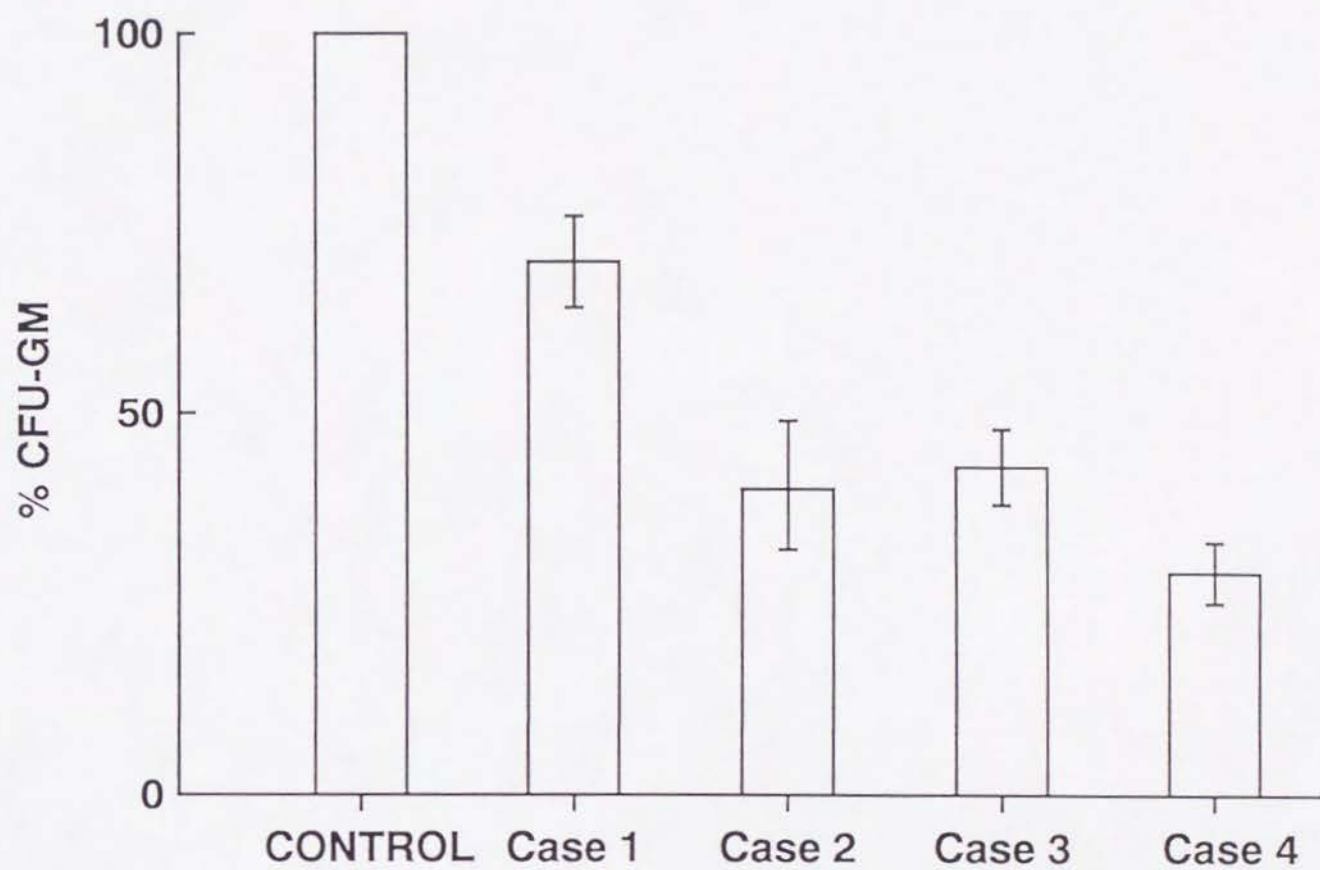
Fig 3

Effect of anti-PGE₂, anti-ferritin, anti-TNF and anti- γ -IFN on CFU-GM inhibitory activity of MDS-CM.

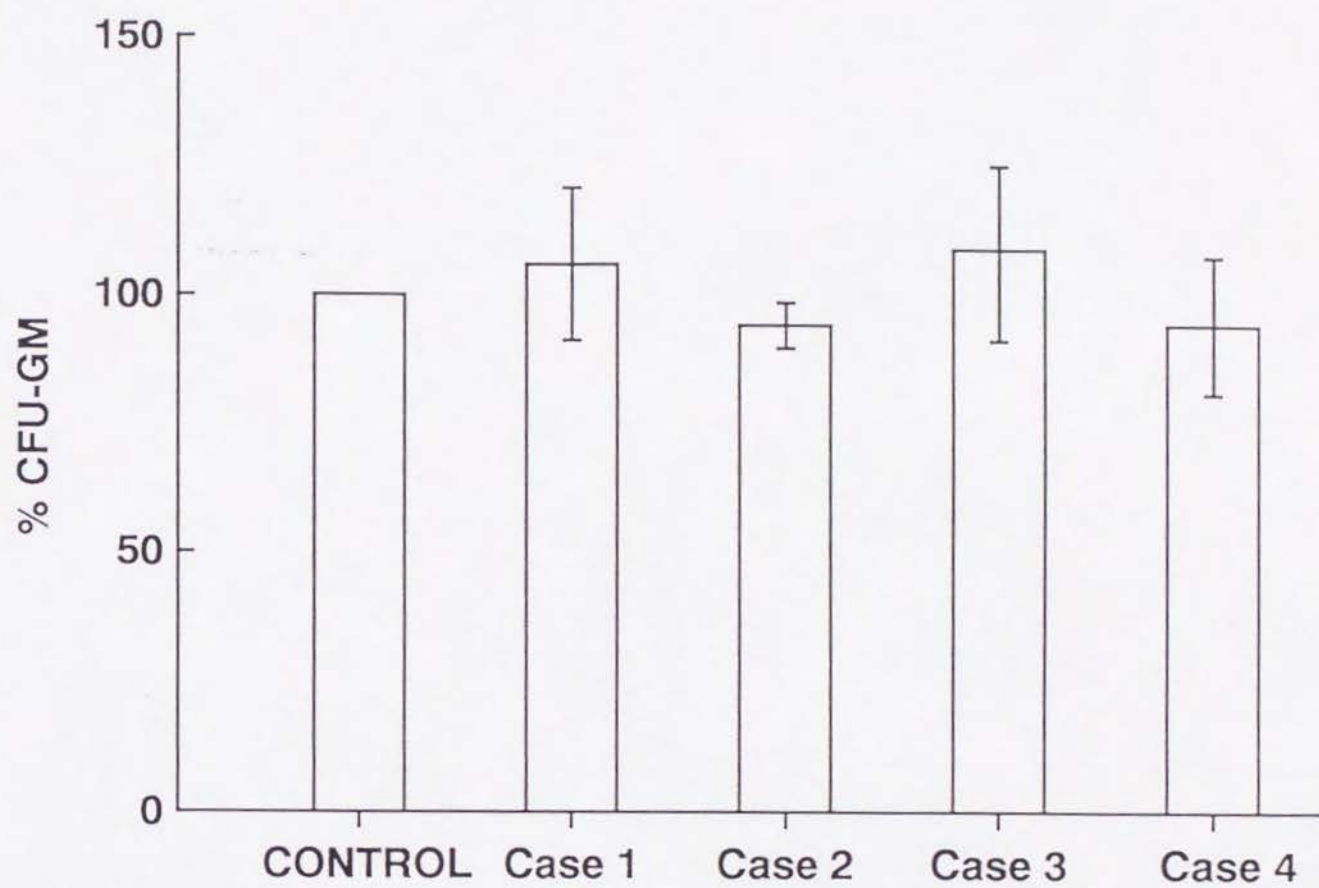
Table 1 Patients Characteristics

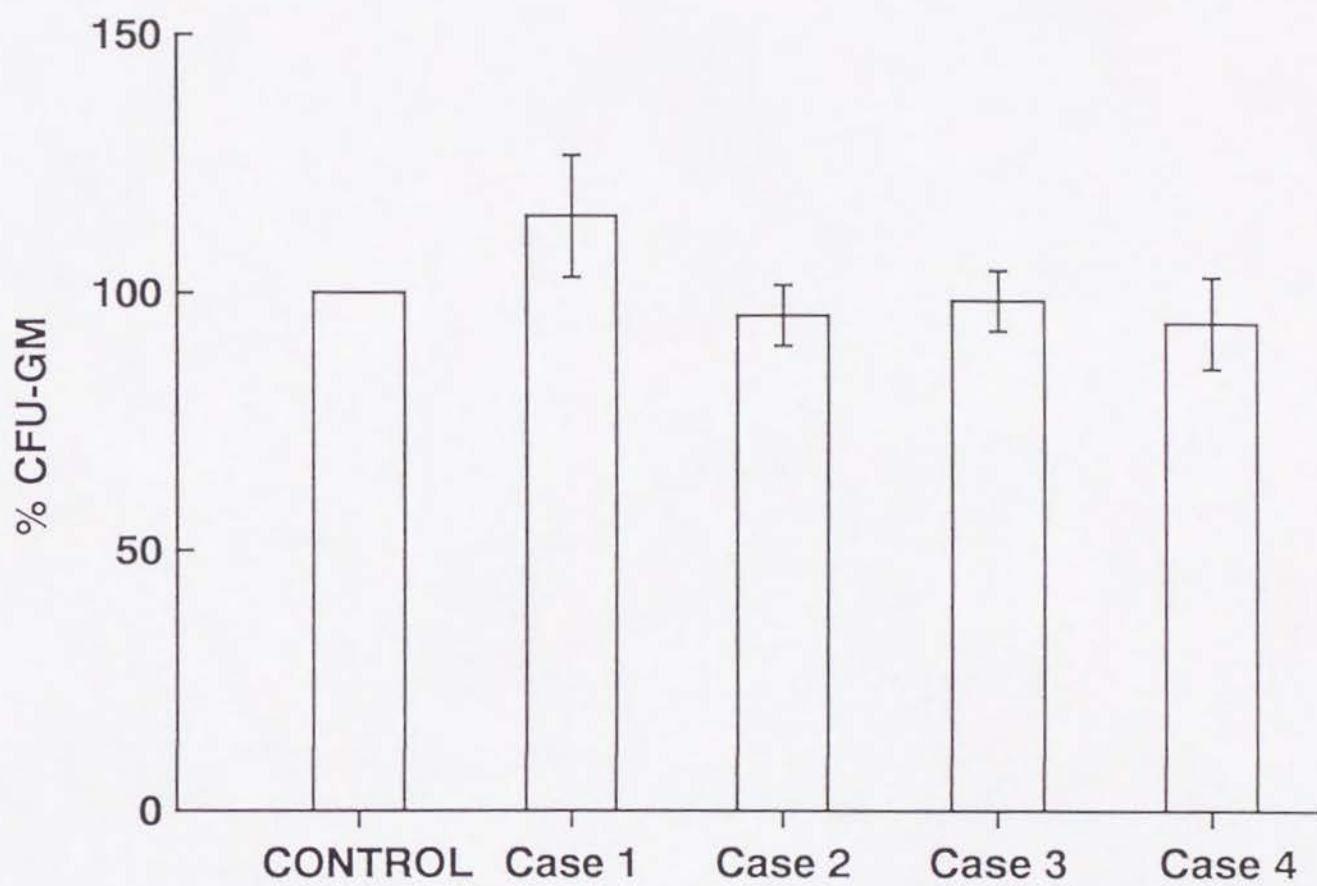
Patient	FAB	Age	Sex	Peripheral Blood				Karyotype	Colony
				Hb (g/dl)	PLT ($\times 10^9/l$)	WBC ($\times 10^9/l$)	Blasts (%)		
1	RA	37	M	9.8	60	3.20	0	47XY,+8	47XY,+8
2	RAEB	60	M	6.8	12	4.20	0	Not Done	Not Done
3	CMMOL	64	M	8.7	71	12.00	2	46XY,-7,der(1),t(1;7)(p11,p11)	46XY,-7,der(1),t(1;7)(p11,p11)
4	RAEB-t	34	M	5.2	72	1.00	48.5	46XY,t(11;19)(q23;p13) /47XY,+13,t(11;19)(q23;p13)	Not Done

F i g . 1

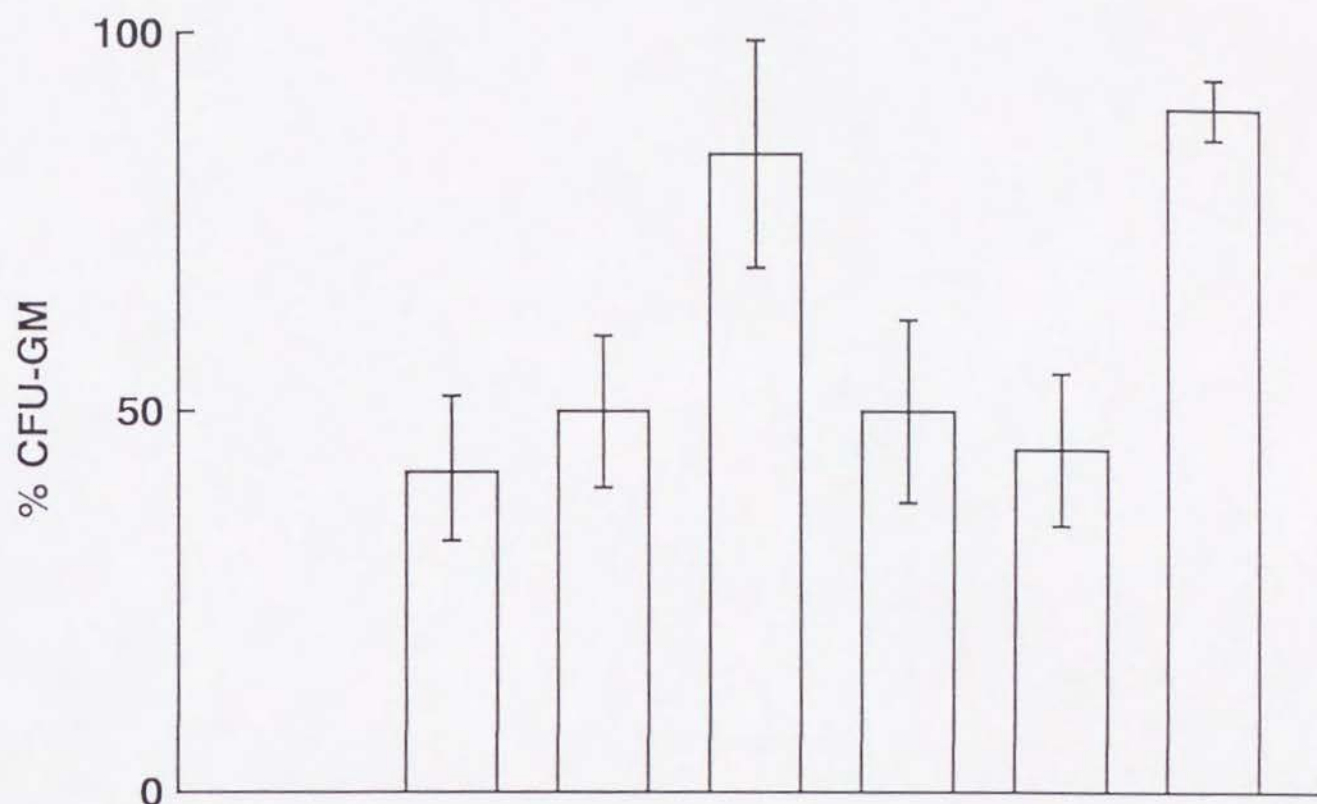


F i g . 2 - A





F i g . 3



(Case No. 3)

MDS-CM	+	+	+	+	+	+
anti-PGE ₂	-	+	-	-	-	+
anti-ferritin	-	-	+	-	-	+
anti-TNF	-	-	-	+	-	+
anti- γ -IFN	-	-	-	-	+	+